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ON EXPRESSED YEAST-CELL PLASMA (BUCHNER'S  
"ZYMASE").

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*Introduction.*—In 1897 a communication was published by Professor E. Buchner\* in which he described a method by means of which he claimed to have isolated for the first time the active alcoholic ferment from the yeast-cell and to have demonstrated its action upon fermentable sugars. Since then Buchner, mainly in conjunction with Rapp, has from time to time given an account of his further investigations in this direction, and these investigations are still in progress.

\* 'Berichte d. deutsch. Chem. Ges.,' 1897, p. 117. *Vide* also succeeding papers, 1897—1900, *ibid.*

These further investigations, Buchner considers, are confirmatory of the conclusion drawn by him from his original experiments, viz., that the activity of the yeast-cell as an alcoholic ferment depends upon the action of a soluble enzyme of an albuminoid character elaborated by the living cell. To this soluble ferment Buchner applies the name "Zymase."

The subject presented so many phases not only of special but also of general biological interest that we were led to pursue its investigation. We considered this the more necessary since Buchner's experiments were carried out entirely with bottom-fermentation yeasts, and it appeared of interest to ascertain whether top-fermentation yeasts (as used in English brewing) give parallel results. At the outset we carefully adhered to Buchner's method of expressing the cell plasma; but owing to the tediousness of the process we were led, after many attempts, to adopt the following arrangement for the extraction of the cell plasma or juice.

*Method of Preparation of the Cell Plasma.*—The yeast as received from the brewery is a thick, pasty, frothy mass, consisting of yeast-cells intermixed with more or less fermented wort. For the purpose, in view it is necessary to separate the yeast-cells from all adherent matter which would by its presence influence the composition of the expressed juices. The purification of the yeast is thus a necessary preliminary operation, and is accomplished as follows:—

To the pasty mass of crude yeast is added an equal part of water and the mixture stirred together. This suspension of yeast-cells is then centrifugalised, whereby the contained cells are separated as a thick creamy mass at the bottom of the containing vessel. The supernatant liquor is decanted and the mass of cells again stirred into a suspension in a fresh quantity of water. The mixture is again centrifugalised, and the process repeated until the last added water comes away clear and colourless. The final product of this process is a firm mass of yeast-cells closely packed together, with a minimal quantity of adherent water. It is necessary to remove even this quantity of water if a natural juice is to be obtained. The pasty mass of yeast is wrapped in a double thickness of "hydraulic chain cloth" and introduced into one of a series of shallow iron trays, so constructed that the pile can be strongly compressed in a hydraulic press and the expressed liquor run off. In this process, which is a modified form of filter-pressing, the last adherent portions of water are removed from the yeast-cell, and the mass of yeast as removed from the cloths appears as a perfectly dry white powder, consisting of yeast-cells with approximately dry exteriors. The pressure necessary to produce this result is from 70—100 atmospheres.

The disintegration of the yeast-cell is the next process. This is accomplished by a mechanical contrivance which maintains the yeast,

together with a proportion of added silver sand, in a condition of violent agitation, in such a manner that in the rapidly succeeding mutual impacts of yeast-cell and sand-particle the cell wall is ruptured and the contents expelled.\*

If the dry mass of yeast and sand be watched while disintegrating it will be seen to become rapidly pasty, and through successive stages of viscosity it finally reaches a perfectly fluid condition. A microscopical examination at the end of the process fails to discover any whole cells. During the continuance of this process, and in fact during the whole time that elapses between the rupture of the first cell wall and the examination of the final product, the material is kept cool by means of a brine circulation. The brine is maintained at a temperature of  $-5^{\circ}\text{C}$ . by means of expanding anhydrous ammonia. This suffices to keep the disintegrating mass at about  $15^{\circ}\text{C}$ . If this precaution is not adopted the temperature will rise to nearly boiling point, owing to the mechanical production of heat by the impacts and friction of the disintegrating mass.

It now remains to separate the escaped intracellular juices from the suspended cell walls. This is accomplished by a repetition of the same process by which the adherent water was removed from the original yeast. To reduce the mass to a consistency capable of being dealt with by the press, kieselguhr is added (Buchner uses this substance together with sand for grinding). The addition of this substance also serves as a filtering material, and allows the expression from the doughy mass—as from a sponge—of a perfectly clear opalescent product in which no suspended particles can be discovered. A pressure of from 200—300 atmospheres is requisite to express the contained fluid. Such are the main outlines of the method which has been adopted in the preparation of the juice on which the observations that follow were made.

It may be useful to give figures representing the method as it operates in practice on an averagely successful preparation.

From 100 grammes of dried and pressed yeast will be obtained from 30—35 c.c. of expressed juice. The weight of sand employed for grinding will be 100 grammes, and the weight of kieselguhr necessary to reduce the ground mass to a suitable consistency for pressing will be about 80 grammes. The specific gravity is usually from 1050—1060, and the time necessary to completely disintegrate the above quantity of dried yeast is usually  $3\frac{1}{2}$  hours.

The physical properties of the juice correspond closely with those described as characteristic by Buchner. The contained proteolytic

\* The precise details of this process, which has been successfully employed for the disintegration of micro-organisms, internal organs, glands and muscle fibres, will form the subject of a separate paper.



enzyme was of a very active character, and produced a rapid digestion of the proteid constituents of the juice.

It occasionally happens that great reluctance is displayed by the juice in leaving the kieselguhr sponge when under pressure. This has most frequently happened with very new yeasts—that is, yeast skimmed from the fermentation vats and used directly for the preparation of the juice. There is some evidence to lead us to suppose that this difficulty is correlated with a similar difficulty which is met with when attempting to prepare as near the living condition as possible an intracellular juice of an organ or tissue. For instance, a liver removed from a dog at the moment of death and at once disintegrated will yield no juice on pressing, even if the pressure be raised to a thousand atmospheres or more; whereas a liver not so fresh will yield its juice without difficulty. That kieselguhr has the power of arresting the passage of certain albuminous bodies can easily be demonstrated. Thus we have found that egg globulins are almost entirely retained in a kieselguhr sponge, and even albumin and serum proteids are retained to a certain extent. It therefore is suggested that the juice that was used in the following work was in every case far removed in nature from the condition in which it existed when alive within the yeast-cell; but on the other hand it is much nearer the living condition than that obtained by Buchner, owing to the fact that he employed water to extract his juice, and water as will be shown has a decided action on the juices we obtained.

We are therefore placed in the difficult position that those conditions in which the juice is nearest its living condition are just those when it cannot be obtained by the convenient method of pressing. Under such circumstances resort must be had to centrifugalising; but the process is tedious in the extreme, and by the time it is completed in all probability the juice has altered in composition. We hope soon to be in a position to overcome this difficulty.

*Properties of the Cell Plasma.*—In the course of our experiments we employed yeast from five different breweries, which we will designate as A, B, C, D, and E. The first three (A, B, and C) were breweries in the London district, D was in the south of England, and E was one of the very few bottom-fermentation breweries in this country. The greater number of our experiments were made with yeasts from A and B, the yeasts from C, D, and E being only used in one or two instances.

From the outset we found that in practically all cases the juice obtained from the yeasts freely evolved gas, both when standing alone and with the addition of sugar. Our results in the latter respect were fully equal to, and in some instances surpassed, those of Buchner.

We were, however, early confronted with the fact that the *auto-* or *self-fermentation* of the juice gave rise to a considerable volume of gas, a

volume which in many cases exceeded that given by the same amount of juice to which sugar had been added. This auto-fermentation apparently escaped the observation of Buchner, who only incidentally refers to it in one of his papers, and who does not appear to have made any correction for the gas evolved from the juice itself in any of his experimental results. The extent to which this fermentation occurs may be seen from the subsequent tables (Tables I and II); in one experiment, for instance, 100 c.c. of the fresh juice gave no less than 2.98 grammes or 1500 c.c. of carbon dioxide. This spontaneous evolution of gas takes place even when the juice is kept at a temperature sufficiently low to maintain it in a solid condition. In all probability the gas which Buchner mentions as being evolved on heating "Zymase" is due to this cause.

In our earlier experiments we determined the carbon dioxide evolved from the juice alone, or from its admixture with sugar, by measuring the volume of saturated salt solution which was displaced by the gas; but later we adopted a modification of Hart's double titration method, in which the carbon dioxide was absorbed by sodium hydroxide solution, and the amount determined by double titration.

In the experiments on the relationship of the carbon dioxide and alcohol formed, we absorbed the carbon dioxide evolved in 33 per cent. potassium hydroxide solution contained in Mohr's potash bulbs. The alcohol formed in these experiments was estimated by distillation and determination of the specific gravity of the distillate, the weight of absolute alcohol corresponding to the gravity of the distillate being then found by reference to spirit tables.

Control experiments were made in all cases—that is to say, when we were determining the amount of carbon dioxide or of alcohol, formed by any juice from sugar, a corresponding quantity of the juice was placed under identical conditions, but without the addition of any sugar, and the amount of gas evolved or of alcohol formed was carefully determined by the same methods as those used in the experiments in the presence of sugar. We employed antiseptics to inhibit the possible action of yeast-cells or other micro-organisms, the nature of the antiseptics used depending on the object of the experiment. The antiseptics principally employed were sodium arsenite, toluol, and thymol, all at the rate of 1 per cent.

In our earlier experiments we employed 40 per cent. of cane-sugar, this being the concentration which Buchner first considered the most favourable; but we subsequently reduced this to 10 per cent., as we found that greater action was obtained with the lesser concentration. In fact, the larger amount of sugar appeared to exercise a retarding influence on the activity of the juice.

*Nature of Results obtained.*—In Table I we give the results of some of our experiments, in which the gas evolved was measured by the dis-



placement of salt solution. The volume of gas evolved is expressed in this and the following tables on 100 c.c. of juice, although the quantity actually used was, as a rule, either 25 to 40 c.c.

It will be noticed that in nearly every instance more gas was obtained from the auto-fermentation of the juice than from the fermentation in the presence of cane-sugar. This was usually the case with the juice from the yeasts of A, C, and D breweries; how far it is due to the distinctive character of the yeasts or to the high sugar concentration we are at present not prepared to say. Another point to be noted is the great variation in the activity of the juice from different samples of yeast. We noticed this throughout the whole of our experiments, but we are unable to correlate it with any of the physical properties of the juice, such as gravity, &c. It will also be seen that by far the greater part of the action is at an end after twenty-four hours, there being either no further increase in the amount of gas evolved, or comparatively little. This we also found common to the majority of our experiments, as will be seen from subsequent tables.

Table I. Volume of Carbon Dioxide evolved by Cell Juice from different Yeasts, with and without the addition of Cane-sugar.

Source of yeast.	Age of yeast from collection.	Carbon dioxide from 100 c.c. of juice.			
		After 24 hours.		After 48 hours.	
		Alone.	With 40 per cent. sugar.	Alone.	With 40 per cent. sugar.
		c.c.	c.c.	c.c.	c.c.
A*	Fresh	520	280	600	472
A*	1 day	240	308	—	—
A*	2 days	133	164	—	—
A*	Fresh	308	186	308	324
A*	Fresh	400	228	400	340
A*	1 day	285	270	285	320
A*	1 day	162	84	162	150
A†	2 days	990	234	990	290
A†	Fresh	90	170	—	—
A†	3 days	760	560	788	592
A††	Fresh	900	2165	—	—
A*	5 days	280	220	—	—
C*	1 day	550	180	—	—
C†	1 day	540	100	—	—
C*	3 days	160	200	—	—
A*	7 days	120	200	—	—
A*	1 day	65	90	—	—

\* Toluol used as antiseptic.

† Sodium arsenite used as antiseptic.

‡ In this experiment 10 per cent. cane-sugar was employed.

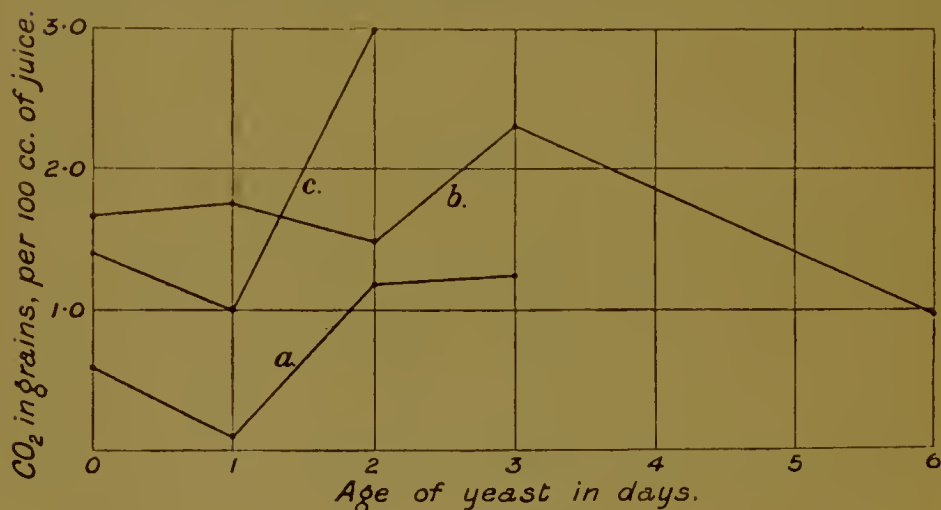
Table II.—Influence of Age of Yeast on Activity of Juice.

Source of yeast.	Age of yeast.	Gas evolved per 100 c.c. of juice.							
		After 24 hours.				After 48 hours.			
		Alone.	5 per cent. C.S.	10 per cent. C.S.	15 per cent. C.S.	Alone.	5 per cent. C.S.	10 per cent. C.S.	15 per cent. C.S.
A.	Fresh.....	grammes. 0·215	grammes. 0·145	grammes. —	grammes. —	grammes. 0·615	grammes. 0·325	grammes. —	grammes. —
	1 day.....	0·105	0·015	—	—	0·120	0·200	—	—
	2 days.....	0·030	0·445	0·395	0·140	1·205	0·965	0·855	0·570
	3 days.....	1·145	—	0·280	—	1·285	—	0·950	—
B.	Fresh.....	1·480	3·030	3·870	—	1·705	3·550	5·060	—
	1 day.....	1·450	3·140	3·770	3·630	1·770	3·590	4·600	4·050
	2 days.....	1·480	3·540	4·410	3·880	1·480	3·920	5·300	3·970
	3 days.....	—	—	—	—	2·310	2·480	6·600	3·300
	6 days.....	0·83	—	1·370	3·230	0·970	—	1·950	3·720
B.	Fresh.....	0·90	2·800	—	3·720	1·420	2·930	—	4·450
	1 day.....	0·97	2·040	4·040	3·590	1·230	2·930	4·440	4·170
	2 days.....	1·32	2·980	3·390	3·150	2·980	3·290	3·700	3·000

*Influence of Age of Yeast on Activity of Juice.*—In Table II we give some of our results on the influence of the age of the yeast, *i.e.*, the time which elapsed between the time of collection of the yeast in the brewery, and that of pressing and grinding, on the activity of the cell plasma obtained. The same table shows to some extent the influence of sugar concentration on the amount of gas evolved.

It will be seen that the results are very variable, but that the general tendency is for the activity of the juice to increase to a certain point with the age of the yeast, the maximum being reached about the 3rd or 4th day from collection. After the maximum is reached there is a very rapid decline in the activity of the juice. The variation in the amount of auto-fermentation is not so great, but the tendency of this is to follow the same direction. The results are shown diagrammatically in figs. 1 and 2, the former showing the auto-fermentation and the latter the fermentation in presence of sugar, both when the gas evolved from the auto-fermentation is included and when it is deducted from the total amount.

FIG. 1.—Showing the influence of age of yeast on auto-fermentation after 48 hours.



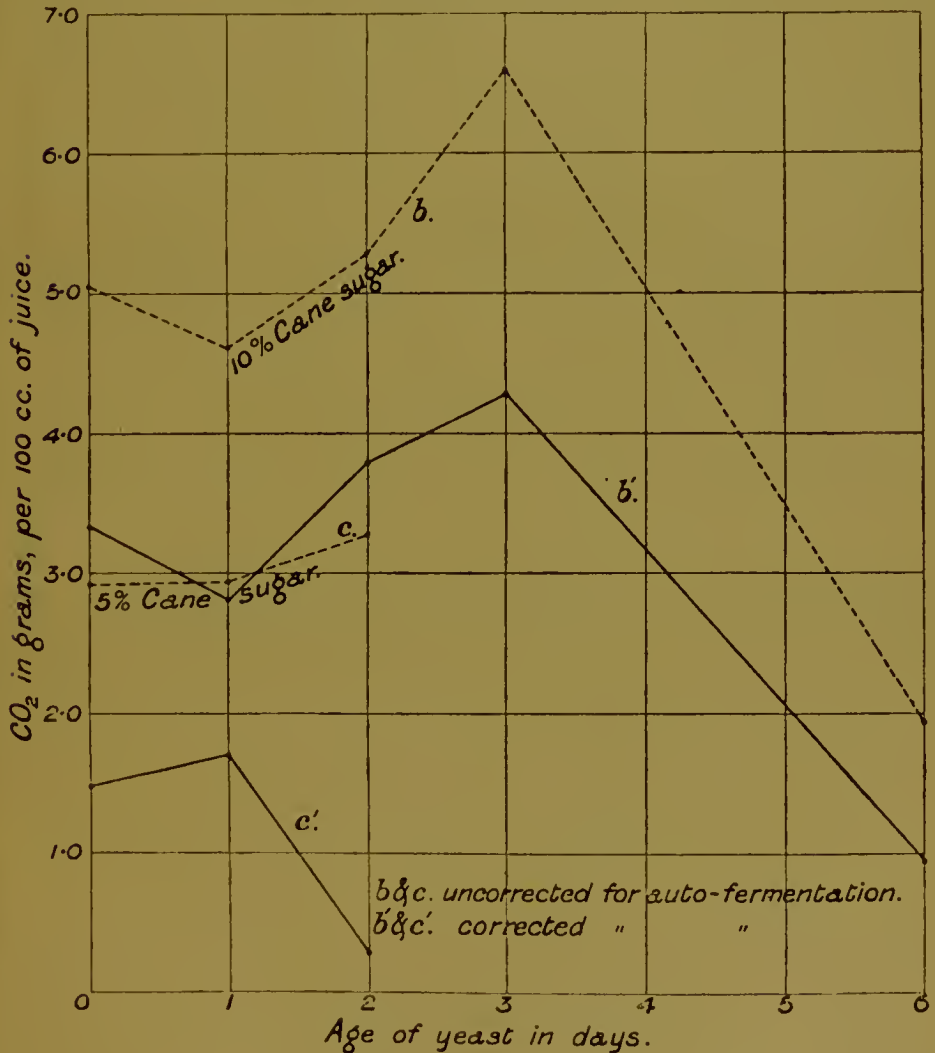
This increase up to a certain point of the activity of the juice with the age of the yeast is the reverse of that which takes place with bottom-fermentation yeasts, as described by Buchner and other Continental observers.

*Influence of Storage on Activity of Juice.*—When the expressed juice is kept even at or below freezing point, its power both of auto-fermentation and of decomposing sugar rapidly diminishes.

*Influence of Amount of Sugar present.*—We carried out a series of

experiments to determine the most favourable concentration of sugar; the results show that the smaller amounts—5 to 10 per cent.—give the most favourable results, whilst the larger quantities sensibly retard the action, *i.e.*, less gas is obtained from the juice plus sugar than from the juice alone. This probably explains to some extent the results we

FIG. 2.—Showing the influence of age of yeast on the activity of expressed juice after 48 hours.

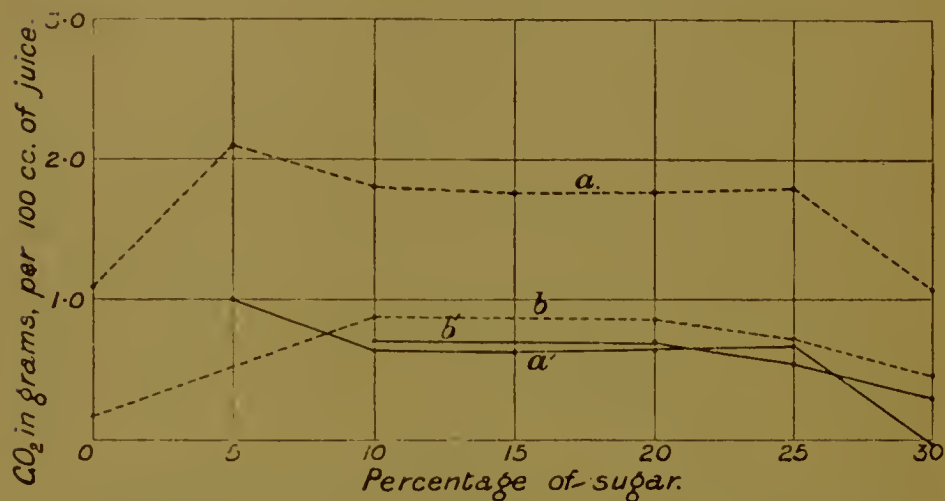


obtained in our earlier experiments in which 40 per cent. of sugar was employed. Some of the results are shown diagrammatically in fig. 3.

*Influence of different Sugars.*—In order to determine if the nature of the sugar employed had any influence on the amount of gas evolved, we carried out a series of experiments with cane-sugar, dextrose, maltose, and levulose at different concentrations, using the same sample of juice

for each set of experiments. The results, as a whole, show that more carbon dioxide is given off from cane-sugar than from either of the other sugars.

FIG. 3.—Showing the influence of different concentrations of sugar on gas evolved.



*a*, After 48 hours (uncorrected); *a'*, corrected for auto-ferm.

*b*, " 24 " " ; *b'*, " " "

*Influence of Temperature.*—We made several experiments to ascertain the most favourable temperature for the action of the juice. As an example of the results obtained, we may quote the following:—

The juice was mixed with 10 per cent. of cane-sugar in the usual way, and there were obtained—

At 0° C....	0.41	gramme of carbon dioxide in 48 hours.
" 10 .....	0.83	" " "
" 25 .....	1.05	" " "
" 37 .....	1.17	" " "

The higher temperatures therefore appear to increase the activity of the juice.

*Influence of Filtration.*—In order to ascertain what influence, if any, filtration through Chamberland and Berkefeld filters had on the activity of the juice, we carried out a series of experiments with different juices, carefully testing their gas-producing activity before and after filtration. Thymol was used as an antiseptic in each case. The results are given in Table III, and it will be seen that filtration decreases to a considerable extent, but without entirely destroying, both the auto-fermentation and the action of the juice on sugar. This decrease in gas-evolving power is accompanied by a very considerable

fall in gravity of the juice. These experiments agree with those of Buchner on the same point.

Table III.—Influence of Filtration on the Activity of the Juice.

Source of yeast.	Age of yeast.	Gravity before filtration.	Gravity after filtration.	Gas evolved from 100 c.c. of juice.			
				Before filtration.		After filtration.	
				Alone.	With 10 per cent. C.S.	Alone.	With 10 per cent. C.S.
	days.			gramme.	gramme.	gramme.	gramme.
B *	3	—	—	—	0·67	—	0·31
B	2	—	—	0·43	0·65	0·00	0·10
B	2	—	—	0·18	1·56	0·25	0·84
B †	2	—	—	1·23	0·83	0·11	0·43
B	2	1055	1018	0·57	0·65	0·24	0·23
B	3	1045	1030	0·25	1·55	0·24	1·17

\* In this experiment the filtration was through a Chamberland filter; in the remaining experiments a Berkefeld filter was used.

† After 72 hours in this case; all the others after 48 hours.

*Influence of Dilution.*—In considering the nature of the action of the juice and of the agent to which the evolution of gas was due, it appeared important to ascertain the effect of dilution on the action of the juice. All experiments were conducted by adding the weighed quantity of sugar to the juice itself, so that no water at all was introduced. If the action were a purely enzymic one, dilution to a limited extent should not appreciably affect the result; whereas if the action were due to other causes, it might be influenced to a greater or less extent. We accordingly carried out a series of determinations on dilution with water alone, with physiological salt solution (0·75 per cent. sodium chloride), and with water in the presence of cane-sugar. The experiments with sugar were made in two ways: in the one, the sugar was added to the juice in the usual way, (10 per cent.), and water was then added to bring about the desired dilution, the ratio of the sugar to juice being therefore kept constant; in the other, the dilution was made with a 10 per cent. solution of sugar, so that the ratio of sugar to the total volume was maintained throughout. The results obtained are set out in Table IV. An examination of the results will at once show that the auto-fermentation of the juice is greatly influenced by dilution both with water and with salt solution. The addition of an equal volume of water sensibly retards the action



Table IV.—Influence of Dilution on Activity of Juice.

Source of yeast.	Age of yeast.	Gas evolved from 100 c.c. of juice.					
		After 24 hours.		After 48 hours.			
		Alone.	Diluted 1 : 1.	Diluted 1 : 2.	Diluted 1 : 3.	Alone.	Diluted 1 : 1. Diluted 1 : 2. Diluted 1 : 3.
(a) With water alone.							
A.....	1 day.....	grammes. 1.49	grammes. 0.02	gramme. 0.00	grammes. 1.71	grammes. 1.32	gramme. 0.05
B.....	5 days.....	0.83	0.54	—	0.97	—	0.58
B.....	5 ".....	1.05	0.00	—	1.37	—	0.09
B.....	6 ".....	1.22	0.31	0.07	—	—	—
(b) With 0.75 per cent. salt solution.							
A.....	Fresh.....	0.90	0.00	0.00	—	—	—
B.....	5 days.....	0.83	0.10	—	0.97	—	—
B.....	5 ".....	1.05	0.00	—	1.37	—	0.08
(c) With water in presence of 10 per cent. cane-sugar on juice.							
B.....	6 days.....	1.30	0.10	0.05	1.30	0.10	0.05
B.....	6 ".....	1.66	0.23	0.08	—	—	—
(d) With water in presence of 10 per cent. cane-sugar on total volume.							
B.....	5 days.....	1.77	0.67	0.47	0.55	—	—

and, in some cases, *dilution with a double volume practically stops the evolution of gas.* With salt solution, the action is still more marked.

In the presence of sugar the retarding action is still distinctly apparent, especially when the concentration of the sugar decreases with dilution. In this case the effect of dilution is fully as marked as in the case of water alone or of salt solution. When the strength of the sugar solution is maintained constant, the retardation is still considerable, but not so great as in the other cases.

This paralysing effect of dilution on the activity of the juice is so contrary to the behaviour of enzymes in general under similar conditions, that in our opinion it forms a grave objection to the acceptance of Buchner's enzyme theory. Since the above experiments were made, we find that Wroblewski\* has conducted dilution experiments with like results.

In connection with the question of the influence of dilution on enzyme action, it may be mentioned that when a sample of six-day juice was diluted to 1 in 1000 with cane-sugar solution, 50.5 per cent. of the cane-sugar was found to be inverted, whilst with another juice, three days old, a dilution of 1 in 100 showed an inversion of 79.5 per cent. of the cane-sugar present. This offers a great contrast to the effect of dilution of the juice on the production of carbon dioxide.

*Ratio of Carbon Dioxide to Alcohol.*—In connection with the question whether we had to do with a true alcoholic fermentation, it became important to determine if carbon dioxide and alcohol were produced in the proportions ordinarily found, and if the amount of sugar which disappeared during the experiment bore any relation to the alcohol and carbon dioxide. We carried out a large number of experiments with a view to elucidate these points, and the results of some of the experiments are shown in Table V. In experiments 1 to 5, the alcohol and carbonic acid estimations were made on the same fermentation, but in experiments 6 to 15 we carried out duplicate fermentations, under identical conditions with the same juice, for the two determinations. We did this in order to ensure greater accuracy in the alcohol estimation, since the escaping gas could be washed by passage through a little water, which was subsequently added to the distillation flask. When we were estimating both products from the same experiment this was not possible.

It will be noticed from the table that the juice as it comes from the press always contains a considerable amount of alcohol, and we found on examination that this agrees fairly closely with the amount of alcohol contained in the yeast, even after the thorough washing and pressing to which it had been subjected in the preliminary treatment.

When corrections are made for the amounts of alcohol and of carbon dioxide formed during the auto-fermentation of the juice, the ratio

\* 'Centralbl. f. Physiol.,' 1899, p. 284.

Table V.—Showing Relation of Alcohol and Carbon Dioxide formed in the Cell-juice.  
The results are expressed on 100 c.c. of Cell-juice.

No. of expt.	Age of yeast.	Original alcohol.	Auto-fermentation.				Fermentation with 10 per cent sugar.				
			CO <sub>2</sub> formed.	Alcohol formed.	Increase of alcohol.	CO <sub>2</sub> formed.	Sugar fermented.	Increase of CO <sub>2</sub> .	Alcohol formed.	Sugar fermented.	Increase of alcohol.
	days	grammes	grammes	grammes	grammes	grammes	grammes	grammes	grammes	grammes	grammes
1	1	1·85	1·38	2·90	1·05	1·64	5·838	0·26	4·75	5·838	1·85
2	2	3·90	1·28	7·90	4·00	0·97	4·292	none	7·90	4·292	none
3	3	3·25	0·65	3·25	none	2·03	6·101	1·37	6·10	6·101	2·85
4	4	4·20	1·89	5·95	1·75	2·45	—	0·56	6·55	4·615	0·60
5	6	4·20	0·95	3·15	none	1·16	1·720	0·21	3·40	—	0·25
6	3	3·70	1·11	4·30	0·60	1·22	2·525	0·11	5·25	2·812	0·95
7	5	3·95	1·20	4·80	0·85	1·21	2·181	0·01	5·25	2·830	0·45
8	7	3·80	0·64	3·80	none	1·09	4·115	0·45	4·25	1·758	0·65
9	7	4·45	0·51	5·05	0·60	1·16	7·413	0·65	5·70	4·390	0·65
10	3	4·12	1·49	4·52	0·40	1·50	3·070	0·01	4·85	2·107	0·32
11	5	2·90	0·40	3·60	0·10	0·59	1·553	0·19	2·75	1·086	none
12	2	2·80	0·73	3·75	0·95	0·91	1·340	0·18	3·90	4·150	0·15
13	1	2·12	0·23	1·75	0·63	0·18	none	none	1·85	none	0·10
14	3	3·15	0·37	3·12	none	2·27	5·041	1·90	5·55	6·413	2·43
15	3	4·17	0·56	4·25	0·08	3·66	8·331	3·10	7·50	9·080	3·25

Experiments 1 to 7 and 12 to 15 were made with dextrose. Experiments 8 to 11 were made with cane-sugar. Thymol was used in all the experiments.

between the residual alcohol and the carbon dioxide is very variable, and only in cases in which a very active juice is employed does the ratio approximate to that found by Pasteur.\* With a weak juice there appears to be little or no connection between the two, the amount of alcohol formed being, as a rule, greater than the carbon dioxide. The small quantities of alcohol to be determined may be thought to be accountable for the discrepancy between the two products, but the following example of a determination carried out in duplicate shows that our methods were capable of considerable accuracy.

	I.	II.
	Gramme.	Gramme.
Carbon dioxide evolved .....	0·327	0·337
Alcohol formed .....	0·95	0·95
Sugar fermented .....	1·167	1·158

One very remarkable fact comes out in all the above experiments, namely, that the amount of sugar which disappears is greatly *in excess* of that actually fermented, as deduced either from the alcohol or from the carbon dioxide formed. The closer the relationship, however, between the two products, the less is the excess of sugar which disappears.

It occurred to us that there might be some constituent of the juice which interfered with the correct determination of the sugar, but we put this to the test and found that when we added sugar to the juice, and then killed its action by heat before any fermentation could take place, the whole of the sugar could be accounted for by Pavy's method of determination.

We also submitted the residual product after fermentation had taken place to hydrolysis with very dilute acid with a view to break up any hydrolysible compound which might have been formed between the constituents of the juice and the excess sugar which had disappeared, but without any result: the reducing power before and after treatment remained the same. The sugar had therefore apparently disappeared as such, and had not simply been rendered unrecognisable to ordinary tests.

We are at present only able to chronicle this most interesting fact, as at the present stage of our work it would be premature to make any theoretical deductions; but in connection with this remarkable disappearance, we venture to throw out the following suggestion:—During the life of the yeast, sugar is consumed by the organism with the resulting production of carbon dioxide and alcohol. Considered in detail, this process probably occurs in two stages—(1) a building up and incorporation of the sugar molecules into the actively living proto-

\* Cane-sugar yields 51·11 per cent. of alcohol and 49·42 per cent. CO<sub>2</sub>.

Dextrose       "   48·55       "       "       "       46·95       "       "

plasm (anabolism); and (2) a breaking down of this complex material into simpler products, of which carbon dioxide and alcohol are the constant and principal constituents (katabolism). May it not be that after the expression of the cell-juice from the cell the same series of actions continues to take place, at least for so long a time as the rapidly changing and unstable cell-juice remains in a condition approximately identical with that in which it existed in the living cell? If this hypothesis be admitted, then the varying activities of the juice are at least partly explicable, for if we designate by  $\chi$  the hypothetical protoplasmic constituent of the cell with which the sugar combines, then we may imagine the processes which take place in the expressed cell-juice (in which we assume  $\chi$  to continue to exist) to be somewhat as follows:—

(a) In the case of auto-fermentation the  $\chi$ -sugar combination, built up during the life of the cell, continues to decompose, after the expression of the juice, yielding carbon dioxide and alcohol.

(b) In the case of the disappearing sugar, the formation of the  $\chi$ -sugar combination continues to a certain point, depending on the activity of the juice, but the decomposition of this combination comes to an end before the whole of the sugar has been liberated in the form of carbon dioxide and alcohol. In the case of a very active juice we may imagine this process to continue until practically the whole of the combination has been decomposed. In the case of a weak juice, the building-up process takes place more rapidly than the breaking-down process, and, consequently, when the activity of  $\chi$  ceases, there remains an excess of sugar in the form of the  $\chi$ -sugar combination.

We are continuing our investigations with the yeast-cell plasma, and shall hope to communicate our further results to the Society in due course. In the meantime it may be convenient to briefly summarise the results we have already obtained, which so far appear to be leading us in the direction not of an enzyme explanation of the process, but rather of a theory which refers the phenomenon to the vital activity of the yeast-cell protoplasm.

- (1.) The top-yeast of English breweries yields, by suitable treatment, a cell-juice which possesses the transient power of decomposing sugar into alcohol and carbonic acid.
- (2.) The amount of gas formed by an active juice is as great as, or even greater than, that found by E. Buchner.
- (3.) The cell-juice as prepared by us undergoes a very considerable auto-fermentation, in some instances exceeding that given by a mixture of the same juice and cane-sugar.
- (4.) A moderate dilution (1 : 2) with water or physiological salt solution practically stops all fermentative activity.
- (5.) Only with a very active cell-juice does the ratio between the



alcohol and carbon dioxide formed approximate to that found in ordinary alcoholic fermentation.

- (6.) When the cell-juice is allowed to act on sugar—either cane-sugar or dextrose—the quantity of sugar which disappears is considerably in excess of that which can be accounted for by the production of carbon dioxide and alcohol.



